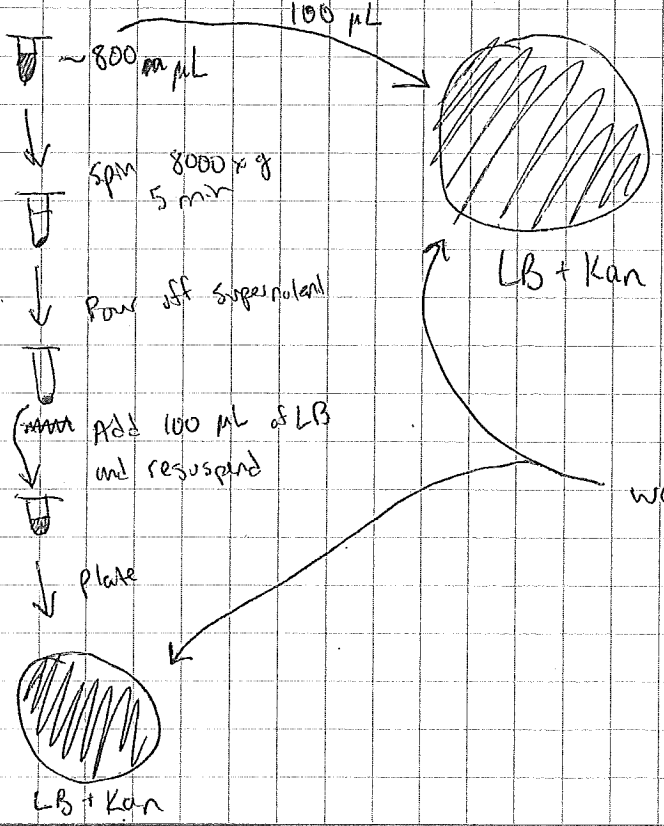
7/13 Ligated the [redacted] vector and insert from the iGEM project. See Kirstie's notebook for specifics on ligation.

After ligation, transformation occurred with the following protocol:

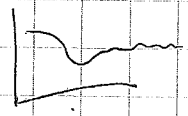
1. Remove competent cells from -80°C freezer
2. Thaw on ice (20-30 min)
3. Add 10pg-100ng (1-5 µL) of DNA to 50 µL of competent cells (used 3 µL each
4. Incubate on ice for 30 min
5. Heat shock at 42°C for 3 min
6. Cool on ice for 5 min
7. Add 750 µL of LB
8. Incubate and shake for one hour
9. Warm selection plates (37°C)
10. Spread 100 µL onto 1 plate (labeled K-B 1 and K-G 1) and spin down the rest at 8000 x g for 5 min.
11. Pour off supernatant, and resuspend pellet in 100 µL of LB and plate (labeled K-B 2 and K-G 2)
12. Incubate plates at 37°C overnight.



Recorded by:	Date	Verified by:	Date

Inoculation ~1:40 pm

SE32
7/14/16
3×3 10mL ASW

Bacteria in reactor aren't ready yet. ~~Mine looks like~~ Curve looks like  and should look like 

So I'll have to wait a bit longer to extract the bacteria

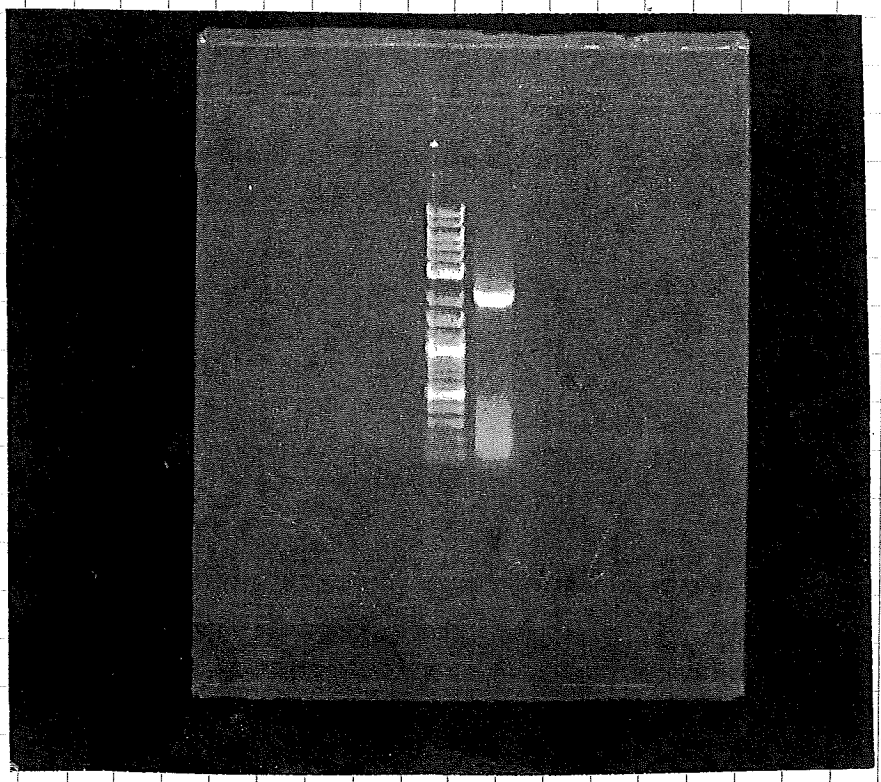
Things to do:

- iGEM
  - ✓ - Take one colony from K-B plate, run ~~MMM~~ colony PCR + streak at the same time
  - ✓ - Run PCR for psB1C3 with higher template ((2.5 µL of each B primer, 6 µL template, 64 µL water, 75 µL Q5) ÷ 3 tubes) 50 µL each
  - Put Maggie and Mitchell's ligations into competent cells (wait until Nick/Gabby/we confirm new competent cells work/are competent).
  - ✓ - Retransform B and G into K with remaining ligation mix from before
  - Run PCR and gel for H
- other
  - ✓ - Take 45 mL ×2 from old reactor and 45 mL ×2 from my new reactor, spin it down, resuspend the pellet, do PCR + run gel to see if I get any better results for identification via reactor solution. (will wait until ~~MMM~~ my reactor is ready so I can do both PCRs at the same time)

d by:	Date	Verified by:	Date

7/18 Repeat transformation of B and G into K. Followed same procedure as on page 45 except used all remaining ligation mix. (~18-20 μL). Let run overnight (labeled Transformation K-B 1A, K-B 2A, K-G 1A, K-G 2A)

Ran PCR for psBiC3 with higher template and got a band ~2 kb



38 min.

(C4) - didn't run C5 and C6 b/c they all the same thing

Good- this means we can ligate and transform into the chloremphenicol (sp.?) vector, which is the one we need for the final submission for the iGEM project.

Need to do PCR cleanup kit on these tubes

TOOK one colony from K-B plate and struck it onto a different plate, then did colony PCR to amplify it and ran a gel to see if it was pres

Post:

PCR cleanup, Nanodrop results:

Tubes labeled as:		Conc. (ng/μL)	260/280	260/230
(C1)	C4	67.7	1.84	1.91
(C2)	C5	366.7	1.39	0.85
(C3)	C6	94.8	1.86	1.97

Recorded by: | Date | Verified by: | Date

7/22 Presentation at UMD on iGEM stuff

[redacted]

7/25 Extracted 45 mL x2 from old reactor (O1 and O2) and from new reactor (N1 and N2), and put back ASW into reactors (sampled from (SE3) for old reactor and my reactor for new).

New was running for ~74 hrs

Old for ~2 months (started 5/26)

Centrifuge down in room in building 30 around the corner (code to get in room is 2/3 + 1). 3500 RCF for 10 min, 10°C.

7/26 DNA never pelleted, which was odd. Decanted N1, O1, and O2 and did a PCR for each with all 4 master mixes after resuspending in 1 mL of nuclease free water (added 10 µL of resuspended DNA to each PCR tube). Used the same PCR protocol as on page 39.

[made LB + chloramphenicol plates yesterday for iGEM, and made iGEM LB + tetracycline plates today]

Designed new primers (will finish tomorrow)

Recorded by: | Date | Verified by: | Date | To Page No.

8/2 Set up PCR for genes A and B using the high fidelity Q5 polymerase and 50 µL reactions

A

25 µL Q5
0.25 µL Forward primer 1
0.25 µL Forward primer 2
0.25 µL Reverse primer
0.5 µL DNA
23.75 µL Nuclease free H_2O

B and B Forced

25 µL Q5
0.25 µL Forward primer
0.25 µL Reverse primer
0.5 µL DNA
24 µL Nuclease free H_2O

B anneal at 72°
BF anneal at ~72°
↳ one at 72° BF1
one at 71° BF2, + BF blank

For hetero Staggered:

A1

25 µL Q5
0.25 µL Forward Primer 1
0.25 µL Reverse primer
0.5 µL DNA
24 µL Nuclease free water

A2

25 µL Q5
0.25 µL Forward Primer 2
0.25 µL Reverse Primer
0.5 µL DNA
24 µL Nuclease free water

anneal at 71°

For A and B (~770 BP), PCR protocol is:

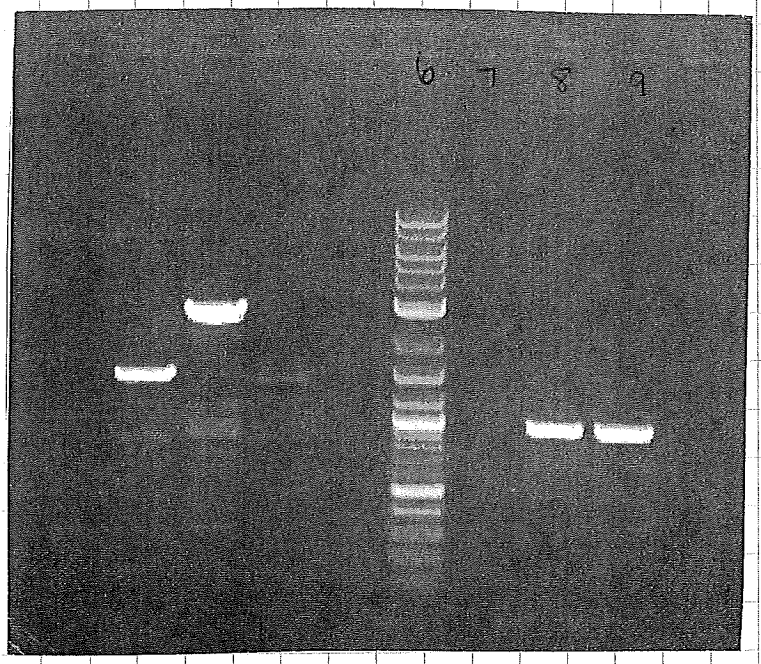
	98°C	30 sec.
↑ 2° ↓ 71° gradient	98°C	10 sec
	[illegible]°C	30 sec.
	72°C	1:30 min
	Repeat × 34	
	72°C	3 min
	10°C	∞

* use Tm Calculator online for Q5 polymerase *

After PCR, do PCR cleanup. Then, combine the two separate tubes into one tube. Heat and slowly cool them to get the desired sticky end

3/3 Ran a gel of some of the PCR product (to the left is Gabby's stuff, can disregard)

Lane	Contained
6	Ladder
7	Blank (BF)
8	BF1
9	BF2

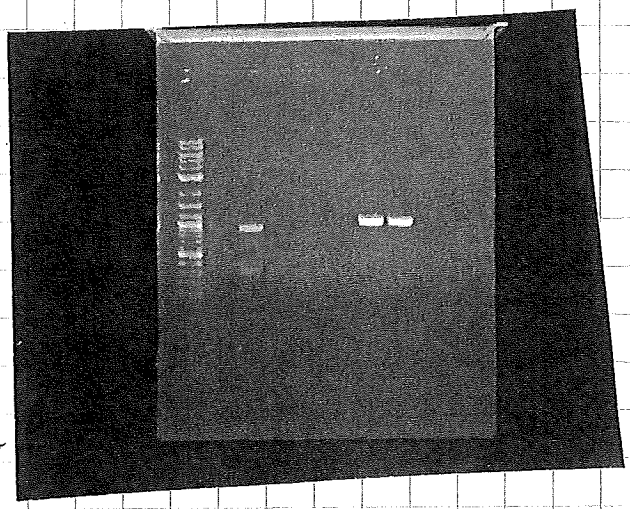


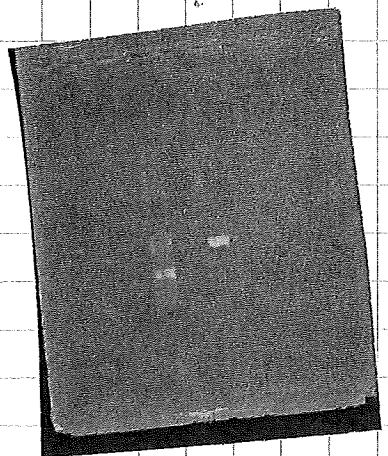
We were able to amplify the forward primer for gene B.

Because of this, we will probably just use the BF tubes for ligations/transformations to avoid the problem of the two start codons in front of the gene using just the BFwd primer. (If this doesn't make sense, look at the primer design document on the computer).

Ran a gel of the rest of the PCR product.

Lane	Contained
1	Ladder
2	Blank A1
3	A1
4	Blank A2
5	A2 (evaporated
6	Blank B
7	B1
8	B2
9	A2 again (still didn't show)
~~10~~	





Ladder, A2 Blank, A2

*Because original A2 tube was cracked, redid it and the blank

To Page No.

3/3 Did a PCR cleanup for A1, A2, BF1, and BF2 (not B1 or B2, as we want use these because of the two start codons before the B gene), and nanodropped.

	Conc. (ng/μL)	260/280	260/230
A1	60.8	1.71	0.96
A2	27.3	1.95	1.72
BF1	30.9	1.96	2.31
BF2	37.9	1.921	1.88

Mixed A1 and A2 into tube labeled A. Used equal amounts of DNA, so took 20 μL of 27.3 ng/μL A2 and 9.0 μL of 60.8 ng/μL A1 (~546 ng DNA each). (29 μL total)

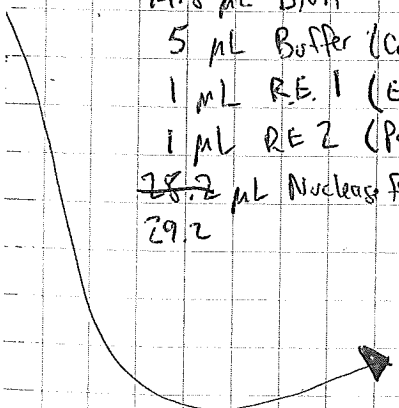
Now a digest will be performed.

Got the previously extracted Chloramphenicol plasmids from freezer (see page 47) and used C1. We want 1 μg of DNA, so:

$$\frac{67.7\ ng}{\mu L} \times \mu L = 1000\ ng$$

14.8 μL of plasmid.

Recipes for Digest (50 μL Reactions)



Plasmid, C1
- 14.8 μL DNA
- 5 μL Buffer (CutSmart)
- 1 μL RE 1 (EcoR1)
- 1 μL RE 2 (Pst1)
- ~~25.2~~ 29.2 μL Nuclease free H_2O

$\frac{1000\ ng}{29} = 37.7\ ng/\mu L$ conc.

Gene A
- 26.5 μL DNA
- 5 μL Buffer (CutSmart)
- 1 μL RE2 (Pst1)
- 17.5 μL Nuclease free H_2O

$\frac{1000\ ng}{37.7\ ng/\mu L} = 26.5\ \mu L$

no RE1 for A because it was heterostaggered to already have the sticky end

* see next page

Gene B (from BF1 and BF2)
- 26.4 μL DNA
- 5 μL Buffer (CutSmart)
- 1 μL RE1 (EcoR1)
- 1 μL RE2 (Pst1)
- 16.6 μL Nuclease free H_2O

$\frac{1000\ ng}{37.9\ ng/\mu L} = 26.4\ \mu L$

To Page No. _____

8/3 After making the digest recipes for each tube, the tubes should be placed in an incubator at 37°C for one hour.

~~We only had 20 µL of BF1 so we adjusted to:~~

Gene B (From BF1)

20.0	µL	DNA
5.0	µL	Buffer (cutsmart)
1	µL	RE1 (EcoRI)
1	µL	RE2 (PstI)
~~23~~ 24	µL	Nuclease free H_2O

Gene B (from BF2)

26.4	µL	DNA
5	µL	Buffer (cutsmart)
1	µL	RE1 (EcoRI)
1	µL	RE2 (PstI)
16.6	µL	Nuclease free H_2O

(added 24 µL H_2O on accident, in case future complications arise)

8/4 Messed up the digestion yesterday, so redid the above procedure

After digestion, a PCR cleanup was performed, then the results were nanodropped:

	Conc (ng/µL)	260/280	260/230
C	15.6	1.96	1.83
A	9.2	2.09	1.69
B	12.6	1.85	1.50

Recorded by: | Date | Verified by: | Date

8/4 Heated and slowly cooled the A tube to get the correct sticky ends from the heterostaggered PCR. Put A in the thermal cycler for the following:

95°C	5 min
85°	30 sec.
75°	30 sec
65°	30 sec
55°	30 sec
45°	30 sec
35°	30 sec
25°	30 sec
15°	30 sec
5°	∞

After this, a ligation was performed for C-A and C-B.
Add the following into a tube:

*ADD LIGASE LAST!

Ligate C-A
2 µL T4 buffer
1.6 µL plasmid C (cut)
3.8 µL A (uncut)
11.6 µL H_2O
1 µL ligase

Ligate C-B
2 µL T4 buffer
1.6 µL plasmid C (cut)
2.78 µL B (cut)
12.62 µL H_2O
1 µL ligase

* See Kirstie's notebook (page 36) for calculations: it was assumed that 25 ng of plasmid K were needed, also 25 ng of plasmid C were needed.

25 ng / 15.6 ng/µL = 1.60 µL C

It was also assumed that since ~35 ng of insert B were needed before, we will still need ~35 ng.

35/12.6 ng/µL = 2.78 µL B

It was assumed that since A is about the same length as B (770 to 771, respectively) ~35 ng of insert A would also be needed

35 ng / 9.2 ng/µL = 3.80 µL A

Nuclease free water was added to bring the volume of each tube up to 20

8/4 After putting the tubes together, incubate them at room temperature for one hour.

After ligation, stored in the freezer until transformation could be done (Monday)

8/8 Transformed AC into competent cells and BC into competent cells, following the same protocol as before (see page 45) except Chloramphenicol plates were used. Let grow overnight and will check tomorrow.

Assuming we successfully inserted the two ArcA genes into two plasmid vectors, we now want to do the same with the two ArcB genes, F and H

Set up a PCR for genes F and H using the high fidelity Q5 polymerase and 50 µL reactions (both will use heterostaggered primers).

F1
25 µL Q5
0.25 µL Fwd Primer 1
0.25 µL Reverse Primer
0.5 µL DNA
24 µL Nuclease free water

F2
25 µL Q5
0.25 µL Fwd Primer 2
0.25 µL Rev. Primer
0.5 µL DNA
24 µL Nuclease free water

H1
25 µL Q5
0.25 µL Forward Primer
0.25 µL Rev. Primer 1
0.5 µL DNA
24 µL Nuclease free water

H2
25 µL Q5
0.25 µL Fwd Primer
0.25 µL Rev. Primer 2
0.5 µL DNA
24 µL Nuclease free water

8/8 Used the Tm calculator to find the temperature for the Q5, which for all 4 is 72°C (F is 1446 bp and H is 1489)

	98°C	30 sec.
↑	98°C	10 sec.
↑	72°C	30 sec.
↑	72°C	1:30 min.
↑	Repeat	× 34
	72°C	3 min
	10°C	∞

8/9 We got no growth on the plates from the transformation from yesterday. Will try again but will use ~~competent cells instead~~ the remaining 17 μL from the ligation (use repeat same protocol as before)
[same protocol as page 45 except use 17 μL instead of 3 μL of DNA in step 3]

There were no bands in the PCR from yesterday, so we repeated the same protocol and will run another gel tomorrow.

8/10 Still no growth on the transformation plates.
Possible reasons for failure:
1. Ligation is wrong
2. Competant cells aren't competant enough
3. There is no antibiotic (chloramphenicol) resistance already in the backbone

We will digest / ligate / transform only the Chloramphenicol vector to see if there is a problem with the backbone or with the plates / concentration of antibiotic.

8/10 Repeated the digestion process from page 56 with Plasmid C (used C3)

Recipe was:

10.55 µL DNA (C3)
5 µL Buffer (cutsmart)
1 µL RE1 (Xbal)
1 µL RE2 (SpeI)
32.45 µL Nuclease free H_2O

* from page 47:

$\frac{94.8\ ng}{\mu L} \times X\ \mu L = 1000\ ng$

$X = 10.55\ \mu L$

After mixing, tube was put in 37° incubator for an hour.

A PCR cleanup was performed, and the results from the nanodrop were the following:

	Conc (ng/µL)	260/280	260/230
C	23.1	2.01	1.42

After cleaned up, the vector was ligated back together.

Ligate C (C L2)

* Add ligase last

2 µL T4 buffer
17 µL plasmid C (cut)
1 µL ligase

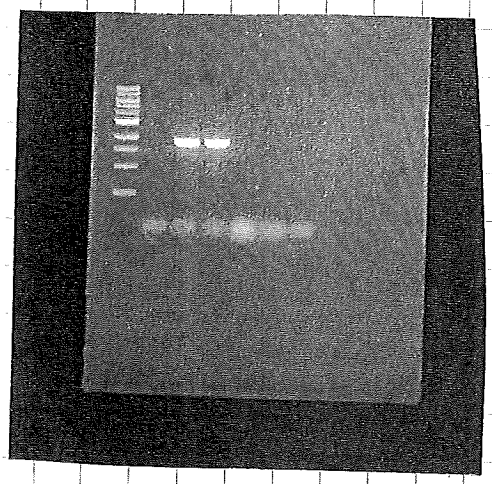
Leave the tube at room temperature for one hour.

After ligation, transformation was performed using the same procedure as on page 45 except chloramphenicol plates were used. Let grow overnight.

Recorded by: | Date | Verified by: | Date

8/10 Ran a gel from the PCR product from yesterday (8/9).

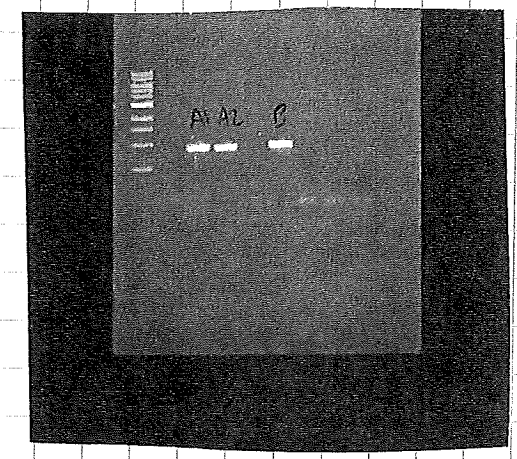
Lane	Contained
1	Ladder (1 KB)
2	F Blank
3	F1
4	F2
5	H Blank
6	H1
7	H2



This time, F1 and F2 amplified, but H still didn't. Will try again.

Redid the PCR for H. Also amplified genes A and B again for future use.

Lane	Contained
1	Ladder (1 KB)
2	A Blank
3	A1
4	A2
5	B Blank
6	B (forced primer)
7	H Blank
8	H1
9	H2



A and B amplified, but H still didn't.

ded by:	Date	Verified by:	Date

8/11 Redid the PCR for H the exact same way, but used a gradient for melting temperature to see if that was the problem. The temperatures were:

H1 - 65°C	H5 - 69.2°C	* tubes labeled as H1A/H1B, H2A/H2B, etc. where A's have Reverse primer 1 and B's have Reverse primer 2
H2 - 65.5°C	H6 - 70.7°C	
H3 - 66.4°C	H7 - 71.5°C	
H4 - 67.7°C	H8 - 72°C	

Ran a gel.

Lane	Contained
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

(See Park's notebook)

8/11 Still no growth on the plates with the transformed plasmid vector.

Looked up the BioBrick Mapper for the vector, and chloramphenicol resistance is built in, so that isn't the issue.

To check if the plates are an issue, we could acquire some bacterial strain that is naturally Chloramphenicol resistant. Tania gave us a strain and I streaked it out today, so we'll see (check with her what exact strain it was, I think it was E.coli ?)

Did a PCR cleanup from the PCR product from yesterday, A1, A2, and B. Nanodropped and got:

	Conc. (ng/μL)	260/280	260/230
A1	17.4	1.81	1.97
A2	20.6	1.99	1.55
B	26.2	1.85	1.43

Mixed tubes A1 and A2 into new tube, A. To get equal ~~concentra~~ amounts of DNA from both, Took 25 μL of A2 ($25 \times 20.6 = 515$ ng) and 29.6 μL of A1 ($29.6 \times 17.4 = 515$ ng) and mixed

Next, we digested.

A
44 μL DNA
5 μL cut smart
1 μL Pst I

$515 + 515 = 1030$ ng
$\frac{1030 \text{ ng}}{54.6 \mu L} = 18.9$ ng/μL

need max. amount because of low concentration, so used 44 μL

B
38.2 μL DNA
5 μL cut smart
1 μL Pst I
1 μL EcoRI
4.8 μL Nuclease free H_2O

$\frac{1000 \text{ ng}}{26.2 \text{ ng/μL}} = 38.2$ μL

To Page No. ____

8/11 Let the digest tubes incubate at 37°C for 1 hour.

After digesting, they were nanodropped. Also got from the freezer the cleaned cut plasmid vector for Kanamycin (K), that Kirstie and I had made before.

	Conc. (ng/μL)	260/280	260/230
A	7.6	2.78	1.75
B	17.7	1.85	0.99
K	12.7	2.09	1.46

Since A is heterostaggered, we had to heat and cool slowly - same protocol as the top of page 58.

Next, A and B were each ligated into K

Ligate A	Ligate B
2 μL T4 buffer	2 μL T4 buffer
2 μL plasmid K (cut)	2 μL plasmid K (cut)
4.6 μL A (cut)	2 μL B (cut)
16.4 μL Nuclease free H_2O	13 μL H_2O
1 μL ligase	1 μL ligase

* Add ligase last

Calculations:

See Kirstie's page 36 for calculations reference

Needed 25 ng of plasmid

12.7 ng/μL × ? = 25 ng

1.97 ≈ 2 μL K

Used 35 ng of B before, so will use that again.

17.7 ng/μL × ? = 35 ng

1.98 ≈ 2 μL B

A and B are the same length, so assumed 35 ng of A were needed too

7.6 ng/μL × ? = 35 ng

4.61 μL A

Recorded by:	Date	Verified by:	Date

8/11 The ligation mixes were left to sit at room temperature for 1 hour.

After this, they were transformed into the gold ultracompetant cells - ~~MMM~~ different protocol than before, Gabby did this, and let grow overnight.

8/12 (My last day at NRL this summer!)

The ~~cells~~ competant cells for A and B finally grew!

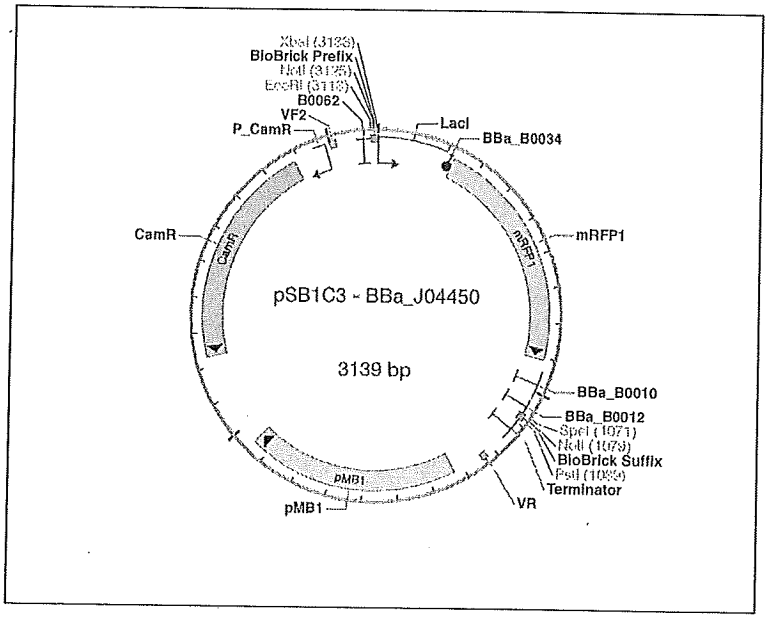
LabGenius

LabGenius BioBrick Mapper

This plasmid viewer is only for the visualization of parts within specified plasmid backbones. It does not show sample availability, or the correct plasmid backbone that a sample may be in.

pSB1C3

RFC #10



Show restriction sites Download map

Synthesize the gene (H) w/ a Gr block set up gradient, same PCR tubes and see maybe new primers w/ ↓ Tm

~~check freeze temp~~

: ~~Maybe~~ get new buffer for digest + ligation

(ATCC - weeks/months to get the bacteria)

F Send email to Nick - does anyone have chlor.-resistant cells

: try ultracompetent cells -- if fail, something wrong w/ steps 1-5

mode of action for chloramphenicol - look up.

~~probably~~ ribosomal

Positive chloramphenicol control strain from Tanya

1. Primers
   - Tm, length, RE sites, hairpins, self-dimers, primer pair dimer
2. PCR
   - Green Mix - Taq
   - Q5 M.M. - Q5 - keep on ice & preheat PCR machine

* PCR cleanup

3. RE digest
   - can't really check well to see if it's working

* Reagents Buffer, enzymes

4. Ligation
   - Buffer - warm & vortex => pipette on ice
   - enzyme
5. Transformation
   - Heat shock
   - cells — use 100 µL, full tube & 20 µl of Ligation
   - plates - use correct plates
6. Plating
   - Drug
   - other chemicals DAP

4. Cycle each reaction using the cycling parameters outlined in Table I. (For the control reaction, use a 2.5-minute extension time.)

TABLE I

Cycling Parameters for the QuikChange Lightning Site-Directed Mutagenesis Method

Segment	Cycles	Temperature	Time
1	1	95°C	2 minutes
2	18	95°C	20 seconds
		60°C	10 seconds
		68°C	30 seconds/kb of plasmid length*
3	1	68°C	5 minutes

* For example, a 5-kb plasmid requires 2.5 minutes per cycle at 68°C.

Dpn I Digestion of the Amplification Products

1. Add 2 µl of the provided *Dpn* I restriction enzyme directly to each amplification reaction.

Notes *Use only the* Dpn I *enzyme provided; do not substitute with an enzyme from another source.*

2. Gently and thoroughly mix each reaction mixture by pipetting the solution up and down several times. Briefly spin down the reaction mixtures and then immediately incubate at 37°C for 5 minutes to digest the parental (i.e., the nonmutated) supercoiled dsDNA.

Transformation of XL10-Gold Ultracompetent Cells

Notes *Please read the* Transformation Guidelines *before proceeding with the transformation protocol.*

XL10-Gold cells are resistant to tetracycline and chloramphenicol. If the mutagenized plasmid contains only the tet^R *or* cam^R *resistance marker, an alternative strain of competent cells must be used.*

1. Gently thaw the XL10-Gold ultracompetent cells on ice. For each control and sample reaction to be transformed, aliquot 45 µl of the ultracompetent cells to a *prechilled* 14-ml BD Falcon polypropylene round-bottom tube.

2. Add 2 µl of the β-ME mix provided with the kit to the 45 µl of cells. (Using an alternative source of β-ME may reduce transformation efficiency.)

3. Swirl the contents of the tube gently. Incubate the cells on ice for 2 minutes.

4. Transfer 2 µl of the *Dpn* I-treated DNA from each control and sample reaction to separate aliquots of the ultracompetent cells.

   As an optional control, verify the transformation efficiency of the XL10-Gold ultracompetent cells by adding 1 µl of 0.01 ng/µl pUC18 control plasmid (dilute the control provided 1:10 in high-quality water) to another 45-µl aliquot of cells.

5. Swirl the transformation reactions gently to mix and incubate the reactions on ice for 30 minutes.

   Note *The incubation time for this step may be reduced to 10 minutes without substantial losses in transformation efficiency.*

6. Preheat NZY$^+$ broth (see *Preparation of Media and Reagents*) in a 42°C water bath for use in step 9.

   Note *Transformation of XL10-Gold ultracompetent cells has been optimized using* ***NZY$^+$ broth.***

7. Heat-pulse the tubes in a 42°C water bath for 30 seconds. The duration of the heat pulse is *critical* for obtaining the highest efficiencies. Do not exceed 42°C.

   Note *This heat pulse has been optimized for transformation in 14-ml BD Falcon polypropylene round-bottom tubes.*

8. Incubate the tubes on ice for 2 minutes.

9. Add 0.5 ml of preheated (42°C) NZY$^+$ broth to each tube, then incubate the tubes at 37°C for 1 hour with shaking at 225–250 rpm.

10. Plate the appropriate volume of each transformation reaction, as indicated in the table below, on agar plates containing the appropriate antibiotic for the plasmid vector.

    For the mutagenesis and transformation controls, spread cells on LB–ampicillin agar plates containing 80 µg/ml X-gal and 20 mM IPTG (see *Preparing the Agar Plates for Color Screening*).

Transformation reaction plating volumes

Reaction Type	Volume to Plate[a]
pWhitescript mutagenesis control	10 µl
pUC18 transformation control	2.5 µl
Sample mutagenesis	10–250 µl[b]

[a] When plating volumes less than 100 µl, place a 200-µl pool of NZY$^+$ broth on the agar plate, pipet the small volume of the transformation reaction into the pool, then spread the mixture.

[b] The optimal amount for spreading varies according to the size and sequence of the mutagenized plasmid. It is generally useful to plate the entire transformation mixture, divided among multiple plates and covering a range of plating volumes.

11. Incubate the transformation plates at 37°C for >16 hours.

Expected Results for the Control Transformations

The expected colony number from the transformation of the pWhitescript 4.5 kb control mutagenesis reaction is >100 colonies. Greater than 85% of the colonies should contain the mutation and appear as blue colonies on agar plates containing IPTG and X-gal.

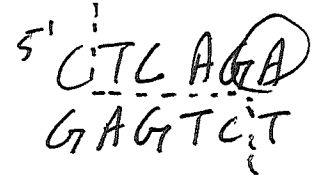
Note *The mutagenesis efficiency (ME) for the pWhitescript 4.5-kb control plasmid is calculated by the following formula:*

$$ME = \frac{\textit{Number of blue colony forming units (cfu)}}{\textit{Total number of colony forming units (cfu)}} \times 100\%$$

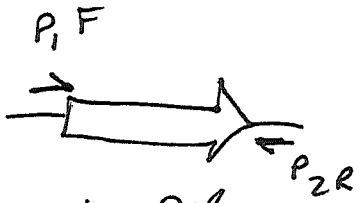
If transformation of the pUC18 control plasmid was performed, >50 colonies (>10^9 cfu/µg) should be observed, with >98% having the blue phenotype.

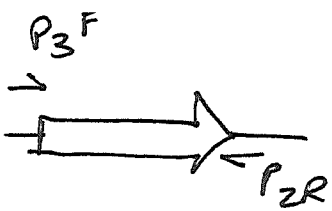
Expected Results for Sample Transformations

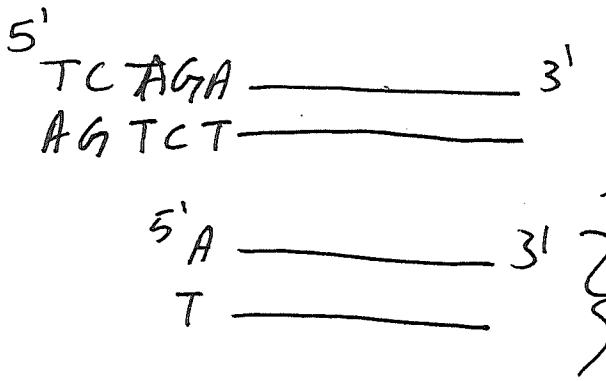
The expected colony number depends upon the base composition and length of the DNA template employed. For suggestions on increasing colony number, see *Troubleshooting*. The insert of interest should be sequenced to verify that selected clones contain the desired mutation(s).



SpeI?







50 ng/µL PCR cleanup

45 ng/µL → Save

→ Heat & slowly cool.

95°C for 30 sec 3 min

Slowly cool to room temp.

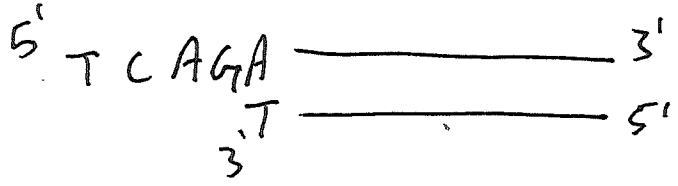
95° 5 mn

~10 30 sec.

~45 reset

5°C ∞

Want is



take 25 µL of the 45 ng/µL and put into tube = 1125 ng

22.5 of the 50 ng/µL put into same tube

47.5 µL ← use for ligation

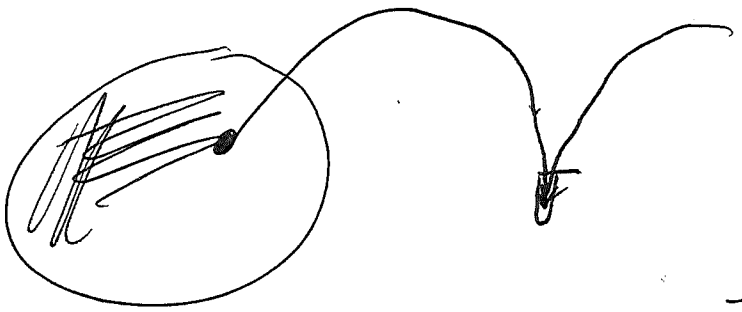
30	bin17_fp	TCCTCGGAATGGTAACGCCGGT	Set 1 Mesorhizobium
31	bin17_rp	AAGTCTGCGGCAAAGCTGGCAT	Set 1 Mesorhizobium
32	bin13_fp	ACGAACTCGTCCTCGTACCGCA	Set 1 Alcanivorax
33	bin13_rp	TAAAACCGCGACCTTCGTGCCC	Set 1 Alcanivorax
34	bin11_fp	ACAGCGCGCGAAAAAGCACAAG	Set 1 Hyphomonas
35	bin11_rp	CTTCGACGGTGCCGTTCAGCTT	Set 1 Hyphomonas
36	bin9_fp	CAGCGATTGCCGCGTTTGATCG	Set 1 Beggiatoa
37	bin9_rp	TGGCGACCGTGTTGATGTCACG	Set 1 Begiatoa
38	bin7_fp	CAAACACGGCGACAAACAGGCG	Set 2 Parvibaculum
39	bin7_rp	CGCAAAACGCATCGGCCACAAA	Set 2 Parvibaculum
40	bin2_fp	CCTCGCTGGCGCTGAAACTGAA	Set 2 Sphingomonadaceae
41	bin2_rp	GTCGCAAGCGCATAAAGCGTCG	Set 2 Sphingomonadaceae
42	labrenzia_fp bin5	GCTGATCGCGCGCTTTGAATGG	Set 2 Labrenzia
43	labrenzia_rp bin5	GTTGCACGCGACGTGAGACTGA	Set 2 Labrenzia
44	tenderia_fp	GTGTTGCAAGCGTGCGTTGAGG	Set 2 Tenderia
45	tenderia_rp	CGCTATGATGCGCGTAACGGGT	Set 2 Tenderia
46	bin18_fp	TCTGCAATTCCCCGTGCAGCAG	Set 3 Muricauda
47	bin18_rp	AGCCATGCGGAACAAACAGGCA	Set 3 Muricauda
48	bin15_fp	CGCGCTGACCAACACCTTGCTA	Set 3 Ruegeria
49	bin15_rp	CCGCGCTCACTTGGCAGAGAAA	Set 3 Ruegeria
50	bin12_fp	TTTTCGGAACCGCGCTGATGCT	Set 3 Parvibaculum
51	bin12_rp	CGAGGCCGCGCGTCTTTATCAT	Set 3 Parvibaculum
52	bin10_fp	GCACGGACTGCAGCTTCTCGTT	Set 3 Alcanivorax
53	bin10_rp	CCCATGGCGCGGTGATCAAAGT	Set 3 Alcanivorax
54	bin4_fp	AAGACGCGGAAGCAGTTCGGTG	Set 4 Alcanivorax
55	bin4_rp	CAAAACCGCTGTTGTCGTGCGG	Set 4 Alcanivorax
56	ander_fp	TGTCGAAGAACTGTGCGACGCC	Set 4 ander
57	ander_rp	CGAGCGGCAACGATACGCTGAT	Set 4 ander
58	Marino_fp	TGCTTGAAGTGCAGCGTCTCGG	Set 4 Marinobacter
59	Marino_rp	GCGGCGATGAATGCGACGAAAC	Set 4 Marinobacter

1
2
3
4

10 tubes

Add Green Mix + Plus water & Primers

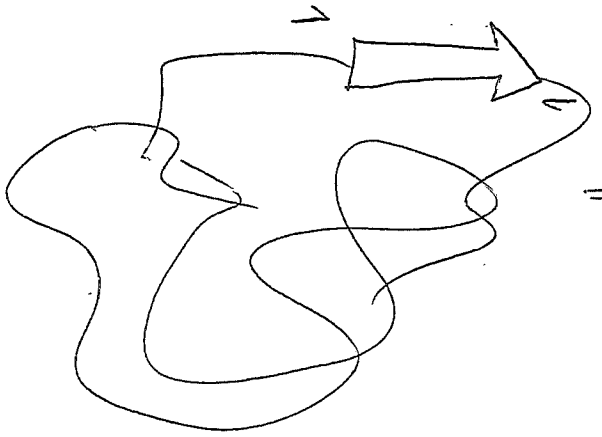
- 12.5 uL Green mix 2X
- .25 uL P_F
- .25 uL P_R
- 12.0 uL H_2O



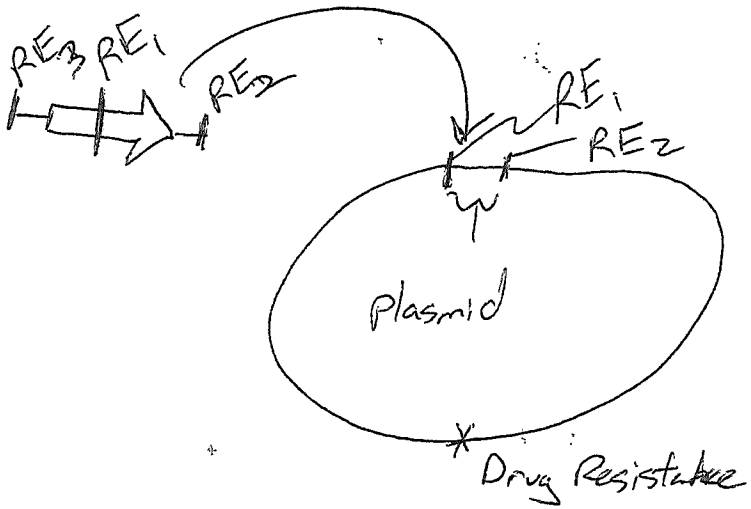
Labrenzia

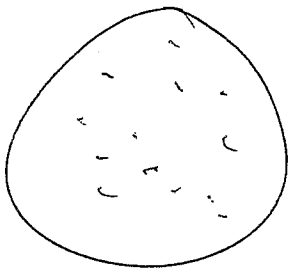
Master Mix x11

137.5 uL of Green Mix
132 uL of H_2O
} 24.5 uL

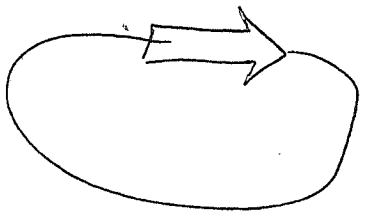


PCR

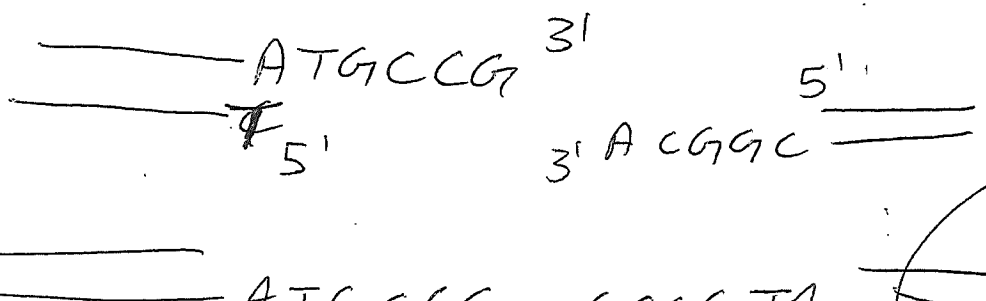

RE2
RE1
RE2
RE1
RE2
plasmid
Drug Resistance

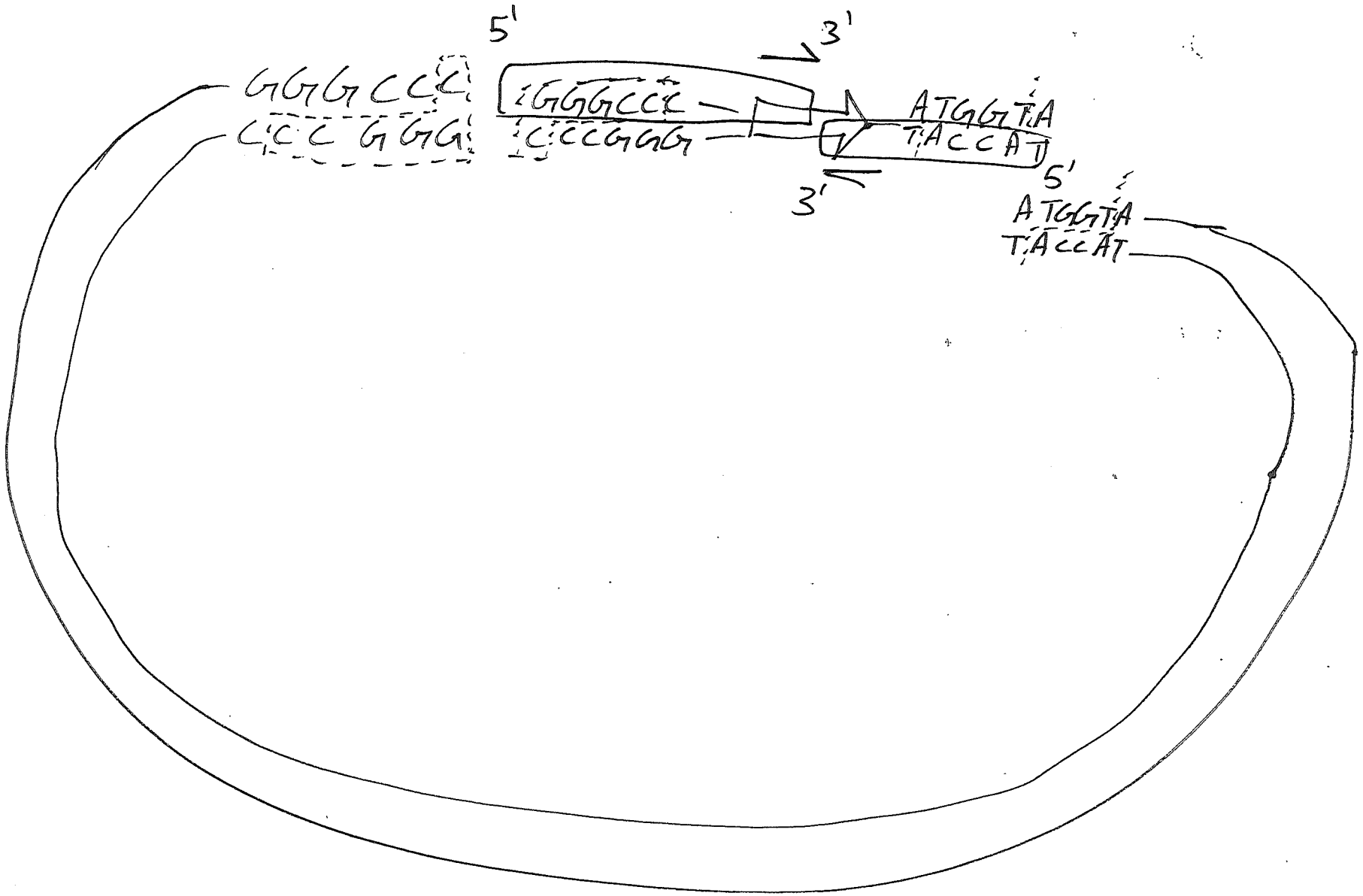


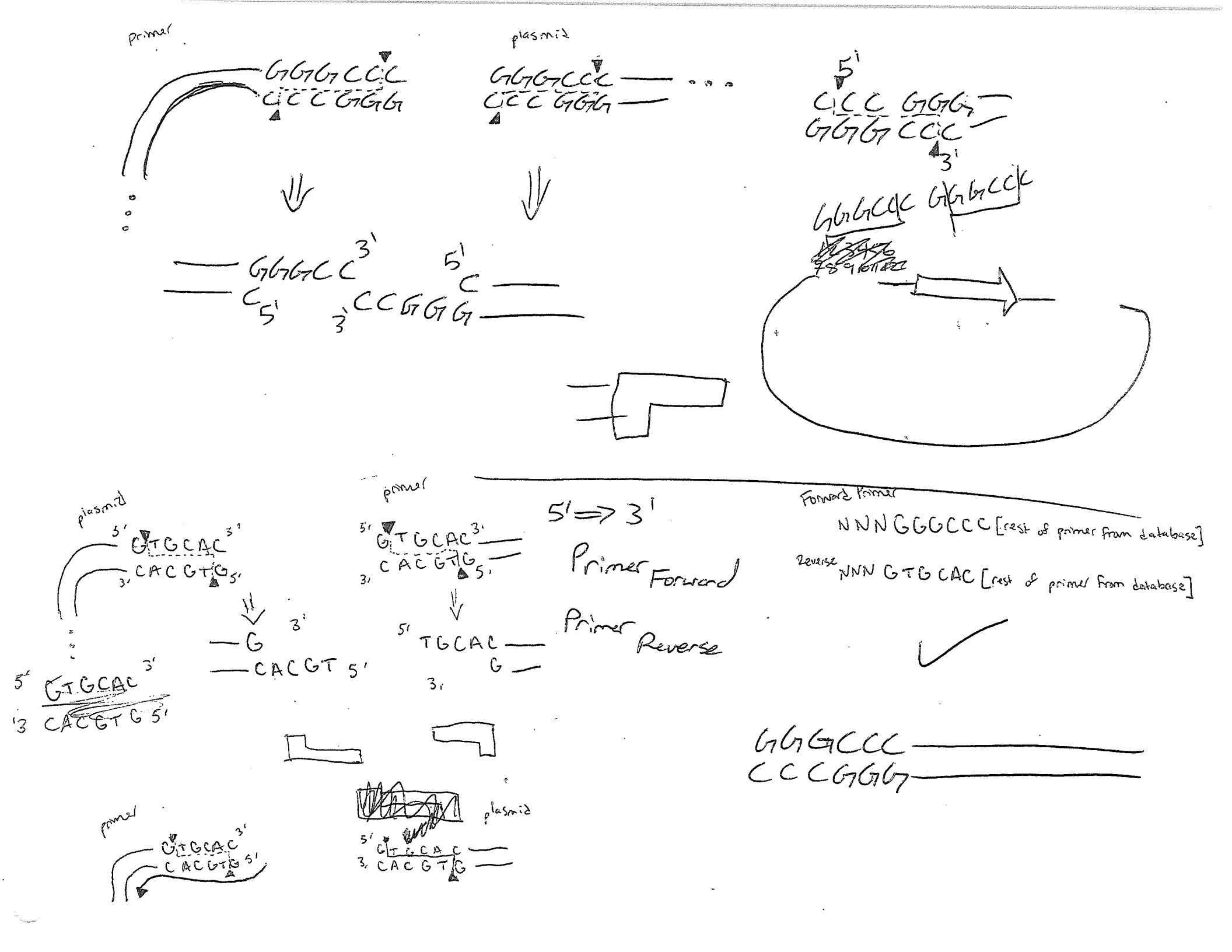
Agar

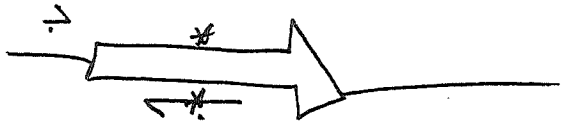


or


ATGCCG 3'
5'
5'
3' ACGGC
ATGCCG GCCGTA
RE1
RE1
Plasmid
RE1
RE1

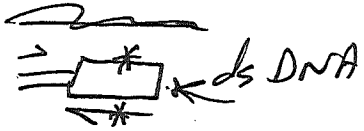

5'
3'
GGGCCC
CCCGGG
GGGCCC
CCCGGG
ATGGTA
TACCAT
3'
5'
ATGGTA
TACCAT

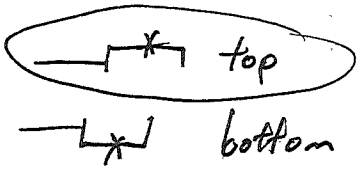

primer
plasmid
5'
3'
Forward Primer
NNNGGGCCC [rest of primer from database]
Reverse
NNN GTG CAC [rest of primer from database]
5' => 3'
Primer Forward
Primer Reverse

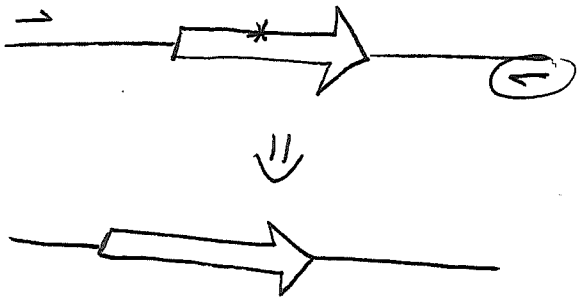


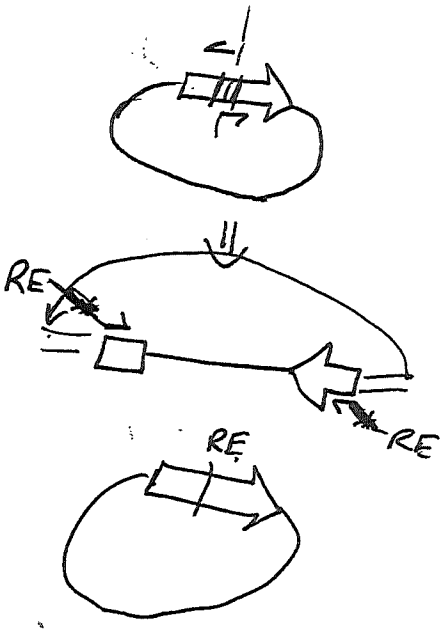
Mega Primers

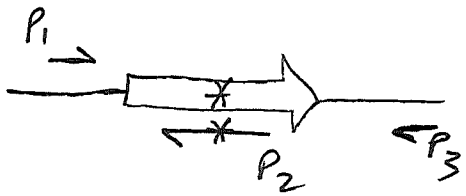
Ultra Primers

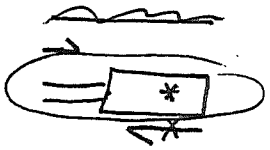




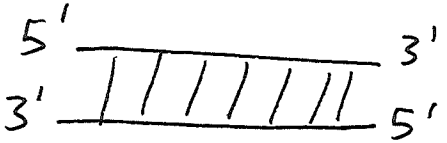




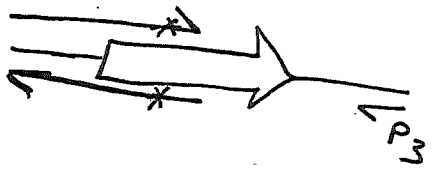


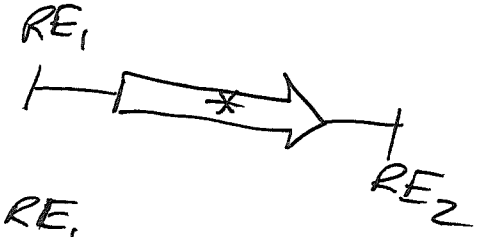


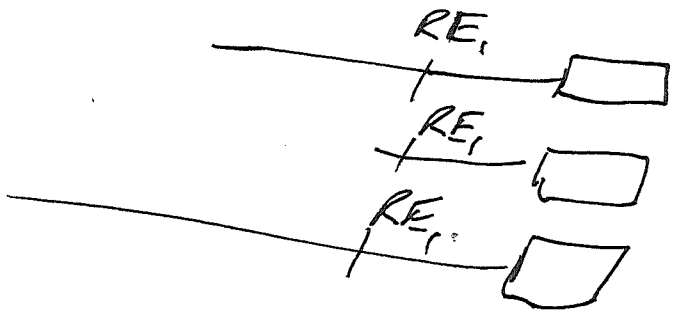
dsDNA + P3



300bp







P
2D